50X1-HUM

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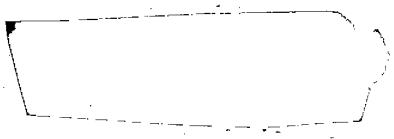
STUDIES ON THE NATURE OF PLANT RESISTANCE TO NEMATODES



Annual Report no.2

Report Period: Aug.1, 1961 - Dec.31, 1962

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Summary

On account of many difficulties existing in the control of plant parasitic nematodes by chemicals or by other suitable methods the significance of the breeding and the cultivation of resistant varieties of various plants cultivated is very great. Therefore, the knowledge of the nature of plant resistance to nematode, or even some approach to do it can have a great significance in breeding work. Besides, investigations carried may also lead to recognition of the existence of chemical compounds in resistant plants, the advantage of which may be found useful in the chemical control of nematodes.

Results of investigations carried out till now on the host-parasite relationship existing between potato and the golden nematode are as follows:

1. Larvae of the golden nematode entered roots of resistant plants /of both investigated hybrids/ in smaller number than those of susceptible plants, this difference being greater in case of the hybrid 56.207/48 than hybrid 56.207/52. Then, the differences in the ability to invade roots of resistant and susceptible plants by larvae do not determine the resistance of potato to the golden nematode.

2. The majority of larvae /ca 90%/ which entered roots of hybrid 207/48 died before reaching higher development stages /above the invasive stage/, in roots of hybrid 207/52 this number being about 50%. Larvae which reached preadult or adult stages were nearly all males /in both hybrids/. Females in higher stages of development were seen only extremely rare. From these results it is clear that the death of larvae in early development stages and the failure to mature females are the main causes of potato resistance to the golden nematode.

3. Larvae which entered roots of susceptible plants caused many changes in various cell elements. In cells affected by nematodes a pronounced decline of chondriosomes, that cell element considered today as producing enzymes in the plant, were seen. Besides and increase in mitotic divisions and cavities in nuclei were observed.

In resistant plants some decline of chondriosomes were found too, but not so pronounced as in susceptible plants. In roots of these plant rather a checking effect on mitotic divisions was seen and no cavities in cell nuclei were found. In plant tissues near the head of the parasite cells with nuclei which looked nearly empty were observed. Sometimes a kind of rods running in cytoplasm from the nematode to the nuclei were seen in these nuclei.

As a result of reactions occuring between golden nematode larvae and cell elements in roots of resistant plants, cells situated close to the nematode die rendering impossible the feeding of nematodes and consequently their death.

4. From preliminary cytochemical studies carried out the supposition can be drawn that nematodes secret some substances which influence changes in cell elements. The reaction of root tissues to these secretions seems to play the main role in the nature of the resistance observed.

The results of investigations carried till now are an approach to the knowledge of the nature of plant resistance to nematodes. Every step made on this way can help us to dissolve the question what kind of properties should have cultivated plants to be resistant to plant nematodes, the control of which is so difficult.

Detailed report

1. Introduction

This is the second annual report covering the investigation period from Aug. 1, 1961 to Dec. 31, 1962. The first annual report covered the period from the beginning of the work, i.e. from Aug. 1, 1960 to July, 31, 1961. In the reporting period investigations started previously were carried further. Works on nematodes invasion of roots of susceptible and resistant plants as well as investigations on changes occuring in cells of root tissues affected by nematodes were carried. To study these phenomena the host parasite relationship existing between potato and the golden nematode was still investigated.

In the previous period it has been established that while larvae of the golden nematode invade roots of both susceptible and resistant potato plants the do it a little faster and in a greater number in susceptible plants than in resistant ones. The work in which a second resistant hybrid /56.207/48/ has been used too, was continued in the reporting period.

Cytological investigations in the previous period have been directed towards establishing the effect of nematodes on chondriosomes. Some observations on changes occuring in other cells elements were made too. Investigations carried in the reporting period were directed to study the influence of nematodes above all on nuclei. The planned works on vacuols could not be started, the majority of time being devoted to study changes in nuclei.

At the end of the reporting period we could begin the biochemical investigations. These investigations were planned to be started earlier but we have had many difficulties in assuring us the help of a specialist. The preliminary investigations which could be started at last have the purpose to know if changes in different cells elements observed till now are connected only with the presence of nematodes /and their secretions/ or if they can be caused by the presence of another

albumen or even by every other substance introduced into root tissues too.

2. Experimental procedures

In works on roots invasion by nematodes the same materials and methods as in previous period have been used. The work was enlarged in that direction that instead of one /56. 207/52/ two resistant hybrid /56.207/52 and 56.207/48/ were used. The work with the second hybrid has been started at the end of the previous period. According to the plan works with S. vernei has been started too, but the plant material obtained from seed of S. vernei was to scanty to by compared in the experiments.

In cytological investigations besides methods used in the previous period the maceration technique to make smears were used too. For this purpose roots were fixed in FAA, Carnoy, AA or Navashin's fluid then macerated with 1 N HCl, pectinase or with a homogenate obtained from potato tuber tissues infected with a culture of Erwinia carotovora /L.R.Jones/ Holland as described in the paper of D r o p k i n [x/] and oth. /2/. Then the material was stained with Aceto Carmine, Acetic orcein, Feulgen or Carmin in propionic acid and mounted in Euparal. Besides smears microtome slides were made too. For this purpose roots were fixed with one of the fixatives mentioned above, embeded in paraffine, sectioned at 15-20 microns, stained with Crystal violet, Haematoxylin Heidenhain or Feulgen.

For biochemical works pieces of tubers were grown in agar medium in Petri dishes and after the roots reached 2-3 cm following substances were injected in them by the aid of a capilary pippette: a/ sterilized water, b/ hen eggs albumen, c/ aqueous suspension of a mass of squashed larvae of H. rostochiensis. At one day's intervals, within 5 days, roots were removed from the agar, fixed, embeded in paraffine, sectioned

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x/ We are very grateful to Mr. V.H.Dropkin, Ph.D., /Nematology Investigations, Crop Protection Research Branch, U.A. D.A., Beltsville, Maryland, USA/ for having kindly send us the culture of Erwinia carotovora.

at 10–15 microns and stained. To investigate the cytoplasm contents coloured reactions were used: for albumen the biuret reaction, for starch – Mc. Manus and J.K.J., for lipids – sudan IV and black Sudan B.

3. Results

The results of investigations on roots invasion of susceptible and resistant plants by golden nematode larvae are summarized in figure 1. The lines on the figure represent only these developing stages of the golden nematode which were found in roots after staining. They do not include females attached on roots surface no males which have finished their development and migrated from roots into the soil. From fig. 1 it is evident, in accordance with our earlier observations, that more larvae /per gram of roots/ entered the roots on susceptible potato plants than those of both resistant hybrids. There are differences between two resistant hybrids too. The hybrid 207/48 appears to be more resistant than 207/52, less larvae entering the roots of the former. The rate of roots invasion was greater after the appearance of first shoots about soil level than before it, this difference being more distinct the more susceptible was the plant.

Roots invasion of susceptible variety and resistant hybrids 207/52 and 207/48 by golden nematode larvae reached its peak at 20, 26 and 23 days after the planting of tubers, the number of larvae /per gram of roots/ being 2130, 1270 and 830 respectively. After reaching their peaks the numbers of nematodes in roots decreased as result of adults development /especially in roots of susceptible plants/ and dying of larvae /in resistant hybrids/.

Lines on figure 2 represent the numbers of larvae in higher stage of development /above the invasive stage/ expressed in per cent of all larvae found in roots at three days intervals after potato planting. Scarcely some per cent of larvae found in roots of resistant hybrid 207/48 have reached further stages of development, after 44 days the number being

11.9%. In roots of resistant hybrid 207/52 and the susceptible variety the corresponding numbers were 47.9% and 96.1% respectively. The majority of nematodes found in roots of susceptible plant 44 after potato planting were developing females. In roots of resistant hybrids developing females were observed only very rarely. Some developing males were seen especially in roots of hybrid 207/52.

In cytological investigations on the influence of golden nematode larvae on the number of mitotic divisions in cells of meristematic regions in susceptible plants within 1 to 2 days after invasion a stimulative effect by the presence of nematodes was observed. Sometimes even twice the number of mitotic divisions occuring in cells of control material /not infected with nematodes/ was observed in cells of tissues infected with nematodes. The nuckei in cells of meristematic region were then much larger than the normal ones and contained more nucleoli /fig. 3/. In these cells the plasma was very dense and rather evently distributed in them /fig. 4/. Some differences in the mitotic division itself in cells of susceptible plants infected with nematodes as compared with control material were seen. Namely, in cells situated close to the head of the nematode chromosomes in metaphase were lying very closely one to another, dispersion of chromosomes to poles was irregular and the telophase was retarded. As however no laggards and no chromosomes bridges were seen, it can be assumed that there were no serious disturbance in karyokinesis.

In hair region /above the meristematic region/ of roots of susceptible plants, within 1 or 2 days after the invasion of larvae, in cells situated in the neighbourhood /3-4 cell layers/ of the head of the nematode an increased nuclei division was observed. This division was not always accompanied with production of new cell walls, resulting in formation of multinucleate cells.

Within 3 or 4 days after roots invasion by larvae in nuclei of cells situated in the neighbourhood of the head of the nematode very often cavities were seen. The first symptom

/possible to see already within 2 or 3 days/ was the formation of small depression on the surface of the nucleus, then a larger fissure was formed and within 5 or 6 days the cavity was gradually enlarging. At the end of the process the cavity was seen to occupy sometimes nearly all the nucleus, small rests of it remaining only /fig. 5/.

In roots of resistant hybrids no stimulative effect on the number of mitotic division caused by the presence of nematodes has been seen. On the contrary, in cells of the meristematic region lying close to the head of ~~the head of~~ the nematode some inhibitory effect /but not a complete check/ was observed: the number of mitotic divisions was here a little smaller as compared with control material. The cytoplasm in cells of meristematic region was not so dense as in susceptible plants and has many vacuoles /fig. 6/. The mitotic division itself in cells of roots invaded by nematodes showed no irregularities as compared with control material /fig. 7/.

In cells of resistant hybrids no such numerous nuclei division within 1 or 2 days as in susceptible plants were observed.

Within 2 or 3 days, near the head of the nematode, cells in which nuclei were only fainty or not at all stained with Feulgen were found /fig. 8/. These nuclei showed no morphological changes /besides that they had dwarfed nucleali/, no depressions and no changes in the thickness of their membranes. They were not mechanically damaged by larvae as they were seen 2-3 cells layers from the head of the nematode. The contents of these nuclei was very poor and they looked empty. In cells of resistant hybrid 207/52 some time nuclei with small cavities were seen. This was not the case in cells of hybrid 207/48.

Sometimes in cytoplasm of these cells narrow rodes which stained intensively red with Feulgen were observed. These rods were lying close to nuclei and even touched them /fig. 8/ or they looked as they were running out from the oral aperture of the nematode. Rarely, in cells with normally stained

cytoplasm these rods were seen too. Till now the nature of these rods was not investigated.

Sometimes, when in roots of resistant plants the meristematic tissues in the appical region were heavy mechanically damaged by strongly moving larvae, a formation of a new meristematic tissue above the injured region were observed.

In preliminary biochemical investigations in roots of susceptible plants after the injection of water or egg albumen a thickenning of cell walls near the point of injection within 2 days were seen. After the injection of the suspension of squashed larvae the formation of large /perhaps giant/ cells rich in cytoplasm and the division of nuclei were observed. Within 3 days after the injection of suspension of larvae in cells lying close to the point of injection a granular cytoplasm gathering around the nucleus was found. The nucleus grew larger, its division began. However, the cytokineze did not occur, only the cell grew larger reaching the size of about 2-3 normal ones /fig. 9/. With coloured reaction it was possible to found in the cytoplasm of these cells small amounts of albumen and lipids, and a lot of starch. However, the longevity of these enlarged cells was very short: already after 4-5 days the granulation of plasma disappeared, the nuclei division stopped.

In cells of resistant plants /hybrid 207/52/ after the injections of water or egg albumen small thickening of cell walls were seen. After the injection of suspension of larvae a heavy thickening of cell walls near the point of injection but no other changes as found in susceptible plants were observed. On the other hand in cells of resistant plants many large starch grain were found /fig. 10/.

4. Discussion

Investigations carried in the reporting period on roots invasion by nematode larvae and on the development of larvae in roots of suscepetible and resistant plants are the continuation of works carried out in the previous report /see annual report Aug.1, 1960 - Jul.31, 1961/. In these investi-

gations, likely as before, we found more larvae /per gram of roots/ both in susceptible and resistant plants after the appearance of the first shoots than before it. However, it seems not likely that these differences are due to such physiological changes in plants after the shoots are exposed to sunlight which make the roots more attractive to the larvae, this suggestion being made by U.S. Department of Agriculture in the comments to progress report no. 3 of this grant. They are rather connected with a greater production of new roots after the appearance of the first shoots, then the stronger production of meristematic tissues suitable for nematode larvae to invade them. We have even observed potato tubers /both of susceptible and resistant varieties/ with shoots developed very late but with roots systems invaded by many larvae.

The decrease of nematode numbers in roots of susceptible plants after they have reached their peaks was due to the development of adults. These adults could not be taken into account the reason being that males have escaped into the soil and many females /cysts/ have fallen off from roots during their washing from soil and the following handling to make suitable preparations for counting larvae in them. In resistant plants the decrease of nematode numbers in roots was caused to some degree by the development of males but mainly by the death of larvae in root tissues. In roots of these plants, it was not possible after some time to identify larvae which have entered into them. Only places where larvae were placed some days ago could be traced as dark spots. It looks as if larvae have been succombed to decomposition.

The phenomenon that most larvae after having penetrate into roots tissues of resistant plants die before reaching adults stages is well known. In our investigations only few larvae reached the third or four development stages in roots of resistant hybrid 207/48, much more in roots of hybrid 207/52 /fig. 2/. The majority of nematodes of other developing stages /above the invasive stage/ found in roots of the last named hybrid were developing males. It seems that the hybrid

207/48 represents a higher degree of resistance than the other one. However, in both hybrids females in fourth /and higher/ development stages were seen extremely rare.

The increased number of mitotic divisions observed in susceptible plants is favourable for nematode development. As a result of this division more plasma is formed which serves as food for the nematodes. On the contraty, in tissues of resistant plants the thickening of cell walls is a disadvantage for the feeding of nematodes.

The observed multinucleate cells in hair region of roots of susceptible plants formed probably later giant cells.

An interesting observations made in roots of susceptible plants was the formation of cavities in nuclei. Sometimes nearly all the nucleus was destroyed. As no mechanical damage of nucleus membrane could be traced, it can be assumed that these cavities were caused by some chemicals secreted by nematodes. In cells of resistant hybrid 207/48 no such cavities were seen, but in hybrid 207/52 sometimes nuclei with small cavities were observed. It seems then that the second hybrid possess a lower degree of resistance than the former, the fact being in accordance with observations mentioned above.

From nuclei which looked empty found in cells of resistant plants probably the chromatine frame and DNA were dissolved /by substances secreted by nematodes/ and washed out as they showed no mechanical damage. My be, rods seen in cytoplasm of these cells, which sometimes were nearly touching the nuclei sometimes looked as if they were running out from oral aperture of nematodes are these secretions? Similar rods have been observed by Dropkin and Nelson /1/.

In roots of susceptible plants no cells which have the appearance to be empty no rods in cytoplasm were seen. In these plants all nuclei were stained well with Feulgen.

The formation of enlarged cells in roots of susceptible plants within 2 days after the injection of suspension of squashed larvae, these changes being not observed after the inject-

ion of water or egg albumen, alows to suppose that these changes were connected with the presence in larval suspension of some subsrances acting like auxins, which caused that the enlarged cells grew secondly meristematic. In connections with these changes many food are directed to the enlarged cells, the synthesis of plasma and nuclei division occur. In our investigations the time of the acting of the stimulus was very short: probably the ammount of secretion injected into the roots with nematode suspension was very small. Therefore after 4-5 days the granular cytoplasm disappeared and nuclei division stopped. However, in roots, when feeding larvae secret substances during a long time the result of it is the formation of giant cells. It is interesting that the contents of in enlarged cells plasma found in our investigations was very similar that of giant cells from the same potato variety.

An interesting phenomenon observed in roots of resistant plants was the presence of many large starch grains in cells lying close to the injection point of larval suspension /fig. 10/. Probably this is connected with an increased production of starch in leucoplasts caused by parasites. Similar observations with respect to plants injured by parasites were made by G u i l l i e r m o n d /3/. My be, there occur reactions which render the change of starch to sugar more difficult decreasing in some degree the possibility to take food by nematodes.

5. C o n c l u s i o n s

Investigations carried out in the reporting period were the continuation of work started previously. It was found that golden nematode larvae invade roots of resistant potato plants in a smaller rate than those of susceptible ones, however, the number of larvae invading roots of resistant plants was high. Then, the differences in the ability to invade roots of resistant and susceptible plants by larvae do not determine the resistance of potato to the golden nematode. The death of larvae in early development stages and the failure to mature females are the main couses of this resistance. Cytological changes

observed in cells elements /mainly in nuclei and partially in cytoplasm too/ confirm the supposition made already in the previous period that the nature of plant resistance to nematode /investigated on potato golden nematode relationship/ is based on differences occuring in cyto- and biochemical reactions occuring between both susceptible and resistant plants and nematodes invading them. Preliminary biochemical investigations seem to point out, that nematodes secret into root tissues some substances which influence changes in cell elements. The reaction of plants to nematodes secretions seems to play the main role in the nature of the resistance observed.

6. Plan for future work

The plan for the following year /Jan.1,1963 - Dec.31, 1963/ include further work based on the host/parasite relationship existing between potato and the golden nematode, namely:

1. Investigations on changes in cytoplasm and vacuoles in roots of susceptible and resistant potato plants after their invasion by nematode larvae.

2. Investigations on factors of a chemical nature taking part in the processes under observation occuring in cells of both susceptible and resistant plants affected by golden nematode larvae.

Literature cited

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3. GUILLIERMOND, A. - 1941 - The cytoplasm of the plant cell. Chronica Botanica Company of Waltham, Mass., USA, 247 pp.

Figures

1.

Numbers of golden nematode larvae /per gram of roots/ found in roots of susceptible potato variety /Dar/ and two resistant hybrids at successive time intervals.

2.

Number of nematodes in higher stages of development /above the invasive stage/ found in roots of susceptible potato variety and two resistant hybrids at successive time intervals, expressed as per cent of all nematodes found in roots.

3.

Smears of meristematic tissue from root of susceptible plant infected with nematodes /ca x 700/. a - nuclei with many nucleoli, Hr - larva.

4.

Transverse section in the meristematic region of a root of susceptible plant infected with nematodes. On can see the dense plasma and nuclei division. a - nucleus with cavitie, Hr - larva.

5.

Longitudinal section in hair region of root of susceptible plant 3 days after larval infection. a - cavities in nuclei, b - the gathered celluloze from dissolved cell walls, Hr - larva.

6.

Transverse section in the meristematic region of a root of resistant plant three days after larval infection. b - necrotic tissue, v - vacuoles, Hr - larva. The plasma is not so dense as in susceptible plants /fig. 4/.

7.

Smears of meristematic tissue from root of resistant plant infected with nematodes. One can see the mitotic division of the nucleus.

8.

Transverse section in the hair region of a root of resistant plant five days after larval infection. b - necrotic tissue, n - empty nuclei, w - rods running in cytoplasm, Hr - larva.

9.

Longitudinal section in the hair region of a root of susceptible plant after the injection of larval suspension /ca x 530/. a - point of injection, b - enlarged cells, n - nucleus, p - granular plasma.

10.

Longitudinal section in the hair region of a resistant plant after the injection of larval suspension /ca x 170/. a - point of injection, b - thickened cell walls, c - large starch grains.